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Anti-*Phytophthora* activity of root extracts from herbaceous species

Efeito inibitório de extratos radiculares de plantas herbáceas na atividade de *Phytophthora cinnamomi*

Ana Moreira*, Isabel Calha, José Passarinho and Ana Sampaio

Instituto Nacional de Investigação Agrária e Veterinária I.P., Quinta do Marquês, 2780-159 Oeiras, Portugal
*(*E-mail: cristina.moreira@iniav.pt)*
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ABSTRACT

The decline in *Quercus suber* (cork oak) and *Q. rotundifolia* (holm oak) "Montado" has been ascribed to the presence of the soil-borne pathogen *Phytophthora cinnamomi*. This oomycete has been considered a relevant agent related to the weakening and death of these oak species, both in Portugal and Spain (Extremadura and Andalucía). The control of this pathogen is rather difficult in the "Montado" ecosystem and the chemical control is not yet efficient. Integrated management strategies are needed to control the pathogen. The inhibitory effect of 15 aqueous root extracts from herbaceous species (Brassicaceae, Fabaceae, Lamiaceae and Poaceae) was tested *in vitro* on *P. cinnamomi* mycelial growth, sporangia and chlamydospore production and zoospore release and viability. The susceptibility of the selected herbaceous species to the pathogen was also evaluated *in vivo* (pot bioassay). Root extracts of some Brassicaceae species can suppress 80-100% *P. cinnamomi* activity *in vitro* showing no susceptibility to the pathogen *in vivo* conditions. The introduction in the "Montado" of a mix of non-hosts herbaceous species with allelopathic effect against the pathogen, incorporated in pastures might be a strategic measure to reduce its population, contributing also to improve soil suppressiveness and quality.

Keywords: Allelopathy, "Montado" ecosystem, oak decline, *Quercus suber*, suppressiveness

RESUMO

O declínio dos montados de *Quercus suber* (sobreiro) e de *Quercus rotundifolia* (azinheira) tem sido associado à presença do patogénio *Phytophthora cinnamomi*. Este oomiceta é um dos principais agentes bióticos associados ao enfraquecimento e morte dos sobreiros e azinheiras, em Portugal e em Espanha (Extremadura and Andalucía). O seu controlo é difícil e o recurso a substâncias químicas não é ainda eficaz, pelo que é necessário encontrar estratégias integradas de gestão. O efeito inibitório de 15 extractos aquosos radiculares obtidos de plantas herbáceas (Brassicaceae, Fabaceae, Lamiaceae e Poaceae) foi testado *in vitro* na actividade de *P. cinnamomi* (crescimento micelial, produção de esporângios e clamidósporos e ainda na libertação e viabilidade de zoósporos). A susceptibilidade à infecção causada por *P. cinnamomi* nas plantas herbáceas seleccionadas foi também avaliada *in vivo.* Os extractos radiculares de algumas espécies de Brassicaceae não hospedeiras de *P. cinnamomi* inibiram em 80 – 100% a sua actividade *in vitro.* A introdução nas pastagens do montado, de misturas de plantas não hospedeiras do patogénio, com efeito alelopático, pode ser uma medida estratégica importante para reduzir a população do patogénio no solo, contribuindo ainda para melhorar a qualidade e supressividade do solo.

Palavras-chave: alelopatia; ecossistema de montado, declínio do sobreiro, *Quercus suber,* supressividade

INTRODUCTION

The decline in *Quercus suber* L. (cork oak) and *Quercus rotundifolia* Lam. (holm oak) "Montados", is a serious problem that has been ascribed to land abandonment, intensification (overexploitation) and inadequate management practices. However, several studies associate the presence of the plant pathogen *Phytophthora cinnamomi* (oomycete) to the weakening and death of these oak species, both in

Portugal and in southern Spain (Extremadura and Andalucía), where a similar situation is observed. The control of this soil-borne pathogen is difficult and its chemical control did not show efficient results till the present. Integrated management strategies are needed to control this pathogen in "Montado" ecosystem. To be applied in extensive areas, these strategies need to consider the tree component and the understory. Research on biological control of forest tree diseases is scarce compared to that developed on herbaceous annual plants.

Considering the need to reduce the use of pesticides as well as the small number of affordable and effective synthetic pesticides, allelochemicals become an alternative to be used in agriculture and forestry. A number of authors point to the clear activity of the allelochemicals as growth regulators, herbicides, insecticides, fungicides and other antimicrobial products, so that in the future they can be used in plant protection (Cheng and Cheng, 2015). The production of secondary metabolites with allelopathic effects is often the result of plant response to stress, acting as plant defense mechanisms against adverse biotic or abiotic factors.

In the Mediterranean flora there are several species with allelopathic effects, such as, those belonging to the families Brassicaceae, Lamiaceae and Poaceae (Araniti *et al*., 2012). Studies on the allelopathic effect of plant species on *P. cinnamomi* are scarce. Castillo-Reyes *et al*. (2015) demonstrated the inhibitory effect, about 70%, of foliar extracts of *Larrea tridentata* (D.C.) Coville (Zygophyllaceae) and *Flourensia cernua* DC (Asteraceae) in the mycelial growth of *P. cinnamomi*.

Biofumigation is a biological control technique widely used in the fight against a wide range of pathogens. The application of this method to control *P. cinnamomi* was studied with 14 species of Brassicaceae, namely *Brassica carinata* A. Braun (abyssinian mustard), *B. juncea* L. (brown mustard) and *B. napus* L. (rape). The most effective in reducing the infection caused by *P. cinnamomi* in roots of *Lupinus* spp. was *B. juncea* (Dunne, 2004; Rios *et al*., 2016). The biofumigation with *Brassica* spp. must be applied in an integrated pest management strategy using a high amount of biomass (*ca*. 7000 kg ha^{-1}) fresh weight (Rios *et al*., 2016).

This study aims to achieve a novel approach to control *P. cinnamomi* enriching pastures with allelopathic species for a sustainable management of "Montado". Our main goal is to obtain efficient plant mixtures to use as pastures in order to reduce *P. cinnamomi* population and improve soil quality and supressiveness in the "Montado".

MATERIAL AND METHODS

Biological material

Fifteen herbaceous plant species (Table 1) with potential allelopathic effect were used for *in vitro* and *in vivo* bioassays. Seeds of each plant species were purchased in commercial houses or collected in the field and sown in pots filled with a soil of volcanic origin (Table 2), under greenhouse conditions at Oeiras.

Root extracts were prepared from 40 days old plants. The inhibitory effect of aqueous root extracts was tested *in vitro* on *P. cinnamomi* structures. The plants were also tested for their susceptibility to *P. cinnamomi* root infection in *in vivo* bioassays.

The *P. cinnamomi* isolates used to prepare the inoculum were Pc 1538, Pc 1539 and Pc 5833, mating

Table 1 - List of plant species studied, identified by scientific name and EPPO Code

Family	Species	Code EPPO*
Fabaceae	*Cicer arietinum* L. 'Kabuli'	CIEAR 'Kabuli'
	Cicer arietinum L. 'Desi'	CIEAR 'Desi'
	Lupinus albus L.	LUPAL
	Lupinus albus L. 'Estoril'	LUPAL 'Estoril'
	Lupinus luteus L.	LUPLU
	Lupinus luteus L. 'Cardiga'	LUPLU 'Cardiga'
Poaceae	*Hordeum murinum* L. (a)	HORMU
	Brachypodium distachyon (*L.*) *P.Beauv.* (a)	BRCDI
	Lolium rigidum Gaud.	LOLRI
	Secale cereale L.	SECCE
Brassicaceae	*Diplotaxis tenuifolia* (L.) DC.	DIPTE
	Raphanus raphanistrum L. (a)	RAPRA
	Sinapis arvensis L. (a)	SINAR
	Brassica nigra L. (a)	BRSNI
Lamiaceae	*Phlomis purpurea* L. (a)	PLMPU

* https://gd.eppo.int; (a) – collected in the field

Table 2 - Soil characterization: the texture components, the main elemental plant nutrients and organic matter

Texture				Mineral and organic characterization					
Sand	Silt	Clay	Texture	K_2O	K	P_2O_5	P	OM	Orgânic N
(%)	(%)	(%)		(mg/kg)*	(mg/kg)*	(mg/kg)*	(mg/kg)*	(g/kg)	(g/kg)
84,8	5,7	5,5	Loamy sand	41	34	25	11	27,8	0,3
				Too low		Low		Medium	n.a.

n.a.- not applied; *- mg / kg (Égner- Riehm)

type A2. The first two were isolated from *Q. suber* collected in 2015 at Miranda do Corvo, Coimbra, and the latter was isolated in 2015 from *Castanea sativa* roots collected at Viseu (both regions localised in the centre of Portugal). The culture maintenance was performed as described by Moreira-Marcelino (2001).

In vitro bioassays – Inhibitory effect

Aqueous root extracts (ARE) of fifteen plant species grown under greenhouse conditions were prepared from roots (10 g fresh weight) in 100 ml of distilled water using a modified method described by Alkhail (2005). A culture of *P. cinnamomi* (isolate Pc 5833) was used in these bioassays.

The *Phytophthora cinnamomi* mycelial growth inhibition test was performed in V8 broth incorporated with ARE at two concentrations (50% and 75% v/v) and the mycelium dry weight (48 hours at 82 °C) was assessed 12 days after incubation at 25 ºC in the dark.

The inhibitory action of ARE on the production of *P. cinnamomi* reproductive structures (sporangia and chlamydospores) was analysed using non-sterile soil extract as described by Moreira-Marcelino (2001) and incorporated with root extracts at 75% (v/v).

The sporangia production and the zoospores release/germination were assessed after six days and the chlamydospores after 12 days of incubation at room temperature (20 °C) without direct light. The quantification of sporangia and chlamydospores was evaluated as the mean number of these structures per mm^2 in the mycelium. Zoospores germination was assessed on Petri dishes filled with V8 agar medium incorporated with ARE at 75% (v/v) and incubated at 25 ºC in the dark.

In vivo bioassays – Susceptibility to P. cinnamomi infection

The susceptibility of plants to *P. cinnamomi* infection was studied in a greenhouse bioassay. These trials were performed during two months between January and March 2017. The plant species, except *Phlomis purpurea*, were tested in pots filled with an artificially infested soil. Inoculum was prepared as described by Moreira-Marcelino (2001) using millet seeds (*Panicum milliaceum* L.) colonized during two weeks with an equal mixture of the three isolates, Pc 1538, Pc 1539 and Pc 5833. To inoculate the pots, 25 g of inoculum was mixed with soil before seeding. The control experiment was prepared in the same way, but using sterilized seeds of *P. milliaceum*.

Ten plants of each species per pot were cultivated in each treatment with five replicates. At the end of the experiment, plant roots were washed with tap water. Confirmation of infection of root tissue by *P. cinnamomi* was assessed by its re-isolation from root fragments in *Phytophthora* selective agar medium P_5ARPH (Jeffers and Martin, 1986) using the procedure described in Moreira-Marcelino (2001). For each plant species, the shoot and the roots were separated and the severity was evaluated by assessing the dry weight of each part (48 hours at 82 °C).

Soil samples from pots were also analysed for *P. cinnamomi* inoculum density. From each pot, in each species, 100 g of soil were collected and pooled to form a homogenous sample that was left to dry for 48h at room temperature. Ten grams of soil per flask, containing 100 mL of 0.2% agar in

sterile water, were shaken and homogenized with a magnetic stirrer during 2 h. A volume of 1 mL of each soil suspension was plated on each of six Petri dishes containing P_5ARPH agar medium. Plates were incubated at 25 °C in the dark. After 24 hours, the plates were washed in running water to remove the soil water agar solution and incubated at 25 °C in the dark for more two days. The plates were then observed under a light microscope. The colonies identified as *P. cinnamomi* based on hyphal morphology (presence of hyphal swellings in cluster together with chlamydospores) were expressed as colony forming units per gram of dry soil (cfu g^{-1}).

Statistical analysis

The percentage inhibition of *P. cinnamomi* was calculated according to the equation: INIB (%) = 100x(C-T)/C. For mycelium growth: C is the mean weight of mycelium produced in the control and T is the mean weight of mycelium produced in the V8 broth amended with ARE. For sporangial and chlamydospore production: C is the mean number of sporangia/chlamydospore observed per mm^2 on mycelium for the control and T is the mean number of sporangia/chlamydospore observed per mm^2 on mycelium in the same substrate incorporated with ARE. For zoospore germination: C is the mean number of colonies for the control and T is the mean number of colonies for the medium incorporated with root extract (ARE) for each species. Data were transformed according to the equation: INIBt = (100 – %INIB)/ 100, where %INIB – percentage of inhibition; INIBt – % inhibition transformed, to satisfy tests of normality. Transformed data were subjected to one way analysis of variance (ANOVA) using the Tukey's test ($p<0.01$) for separation of means.

Statistical analysis was performed using software R 3.3.

RESULTS

In vitro bioassays – Inhibitory effect

Results showed that root extracts obtained from some tested species have a strong anti-*P. cinnamomi* activity with significant inhibition on the mycelial growth and on the production of reproductive structures. The pH of the ARE ranged from 5.6 to 7.2 in all the experiments.

The aqueous root extract of two Brassicaceae species, *Diplotaxis tenuifolia* and *Raphanus raphanistrum,* inhibited significantly the mycelial growth of *P. cinnamomi* on both concentrations tested ($p<0.01$). The mycelial growth inhibition by *D. tenuifolia* ARE ranged from 69 to 83% for concentrations of 50% and 75%, respectively. For *R. raphanistrum* the inhibition ranged from 20 to 56% for the same concentrations (Figure 1A).

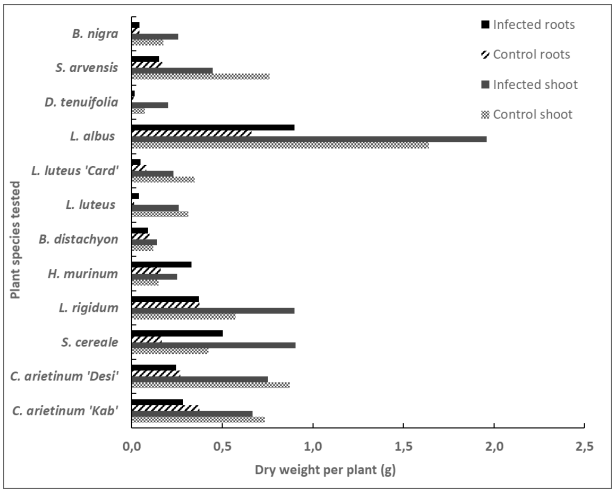

Figure 1 - Mean dry weight per plant (shoot and root) for each species cultivated in infested and non-infested soil with *Phytophthora cinnamomi*

Significant inhibition (81-100%) of sporangia production was observed for the majority of ARE tested (Figure 1B). Three Poaceae species showed different behaviour. For *S. cereale* and *B. distachyon* the inhibition achieved was 36% and 53% respectively, while *Lolium rigidum* ARE showed a significant increase on sporangia and chlamydospores production, which resulted in a high release of zoospores (mean of $4.25x10^5$ mL^{-1}) in compared to the control.

Zoospore differentiation and release were observed on ARE obtained from the same seven species (*B. distachyon, B. nigra, H. murinum, L. luteus, L. rigidum, S. arvensis* and *S. cereale*) which induced sporangia production in a quite different amount

(Figure 1D). The zoospore viability was tested in the presence of all ARE and only four of them showed inhibition in the germination (Poaceae: *B. distachyon* – 59.2% and *S. cereale* – 64.2%; Brassicaceae: *D. tenuifolia* – 100 % and *R. raphanistrum* – 99.4%). (Figure 1D).

Chlamydospore production was also influenced by ARE from *D. tenuifolia, R. raphanistrum, C. arietinum* 'Kabuli', and *C. arietinum* 'Desi'. The other root extracts showed no inhibitory effect; on the contrary, they improved the chlamydospore production (Figure 1C), but their viability was not tested.

***In vivo* bioassays – Susceptibility to *P. cinnamomi* infection**

Figure 2 summarizes the evaluation of plants in the susceptibility tests. Dry weight of shoots and roots were evaluated. The biomass dry weights of seven plant species, namely *L. albus, D. tenuifolia, H. murinum, L. rigidum, S. cereale, B. nigra* and *B. distachyon*, cultivated in the soil infested with *P. cinnamomi* was higher than those of the control (Figure 2).

Table 3 - Quantification of *Phytophthora cinnamomi* in the soil, in the roots of different plant species and the phenological stage of plants

Plant species	Control			Infested soil				
	Phenological stage	Symptoms		Phenological stage	Symptoms		Infected roots	Soil
	BBCH	Shoot	Root	BBCH	SShoot	Root	(%)*	cfu/g**
Cicer arietinum 'Kabuli'	4	No Symp	No Symp	4	No Symp	No Symp	0.0	122
Cicer arietinum 'Desi'	4	No Symp	No Symp	4	No Symp	No Symp	0.0	368
Lupinus luteus	1	No Symp	No Symp	5	Foliar chlorosis and necrosis dead plants	Necrosis	22.2	500
Lupinus luteus 'Card'	1	No Symp	No Symp	4	Foliar chlorosis and necrosis; dead plants	Necrosis	44.4	250
Lupinus albus	2	No Symp	No Symp	3	No Symp	No Symp	0.0	68
Secale cereale	3	No Symp	No Symp	3	Lodging; foliar chlorosis	No Symp	0.0	255
Lolium rigidum	3	No Symp	No Symp	3	Lodging; foliar chlorosis	No Symp	0.0	20
Hordeum murinum	3	No Symp	No Symp	3	Lodging; foliar chlorosis	No Symp	0.0	2
Brachypodium distachyon	3	No Symp	No Symp	3	Lodging; foliar chlorosis	No Symp	0.0	22
Diplotaxis tenuifolia	4	No Symp	No Symp	5	No Symp	No Symp	0.0	30
Sinapis arvensis	5	No Symp	No Symp	5	No Symp	No Symp	11.1	20
Brassica nigra	2	No Symp	No Symp	2	No Symp	No Symp	5.6	85

*Percentage of infected roots by P. *cinnamomi*; ** cfu-colony forming units per g of dry soil (cfu /g) (mean)

In infested soil, the Fabaceae species, *C. arietinum* 'Kabuli', *C. arietinum* 'Desi' and *L. albus*, did not present any disease symptoms in the aerial part. The leaflets showed a dark green color without discolorations. Also, no lesions or necrosis were observed on roots and *P. cinnamomi* was not isolated from them. On the contrary, plants of *L. luteus* showed symptoms of foliar chlorosis and necrosis and *P. cinnamomi* was isolated from their roots. The roots of *L. luteus* and *L. luteus* 'Cardiga' presented necrosis and a lower number of nodules than the other Fabaceae. The total biomass of the infected plants (root and shoot) was lower than the control plants (Figure 2). Additionally, some infected plants died (*L. luteus* – 68% and *L. luteus* 'Cardiga' – 44%).

For Brassicaceae, plants grown on infested soil showed no chlorosis or discoloration in shoot; however, flowering occurred earlier than in the control. The root system was short, thin and brittle, showing no lesions or necrosis, and no *P. cinnamomi* was detected. In infested soil, plants of *D. tenuifolia* showed higher development than control plants (Figure 2), as already mentioned. On the contrary, other species, namely *S. arvensis* and *B. nigra*, were also infected with *P. cinnamomi*, showing that they are hosts of the pathogen.

With regard to the Poaceae plants, a higher tillering was observed in the plants sown in the infested soil, which may indicate a strategy of survival, but no symptoms on roots were observed.

The cultivation of Fabaceae and *S. cereale* increased the potential inoculum of *P. cinnamomi* in the soil. The higher number of propagules was observed in the soil where plants of *L. luteus* 'Cardiga' and *L. luteus* were cultivated, 250 and 500 cfu/g, respectively. This species increases the *P. cinnamomi* inoculum density in the soil.

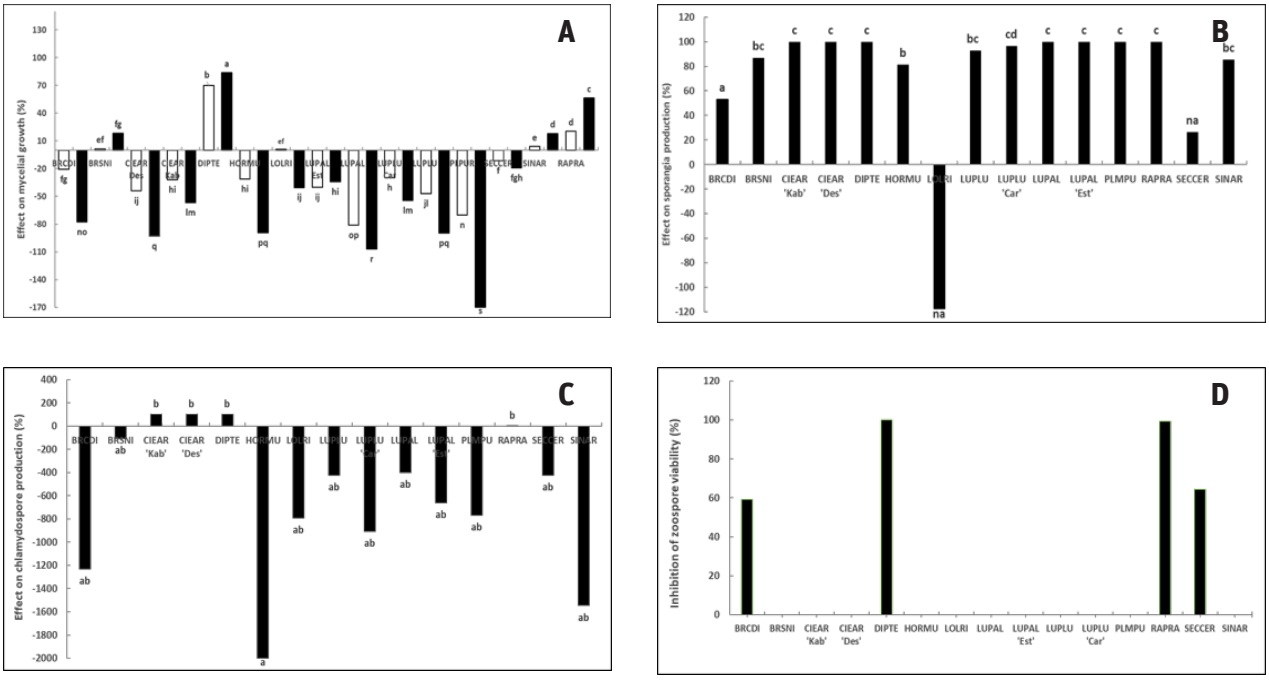

Figure 2 - Effect of aqueous root extracts (ARE) of different plant species on *Phytophthora cinnamomi* activity assessed *in vitro*: A-mycelial growth (%); B- sporangia production (%); C- chlamydospore production (%); D-zoospores germination (%); Plant species: BRCDI-*Brachypodium distachyon;* BRSNI-*Brassica nigra;* CIEAR 'Desi'- *Cicer arietinum* 'Desi'; CIEAR 'Kab'-*Cicer arietinum* 'Kabuli'; DIPTE- *Diplotaxis tenuifolia;* HORMU- *Hordeum murinum ;* LOLRI- *Lolium rigidum* ; LUPAL 'Est'- *Lupinus albus* 'Estoril'; LUPAL - *Lupinus albus*; LUPLU 'Car'-*Lupinus luteus*'Cardiga'; LUPLU -*Lupinus luteus* ; PLMPU- *Phlomis purpurea*; SECCE- *Secale cereale*; SINAR-*Sinapis arvensis*; RAPRA-*Raphanus raphanistrum*; Bars topped by the same letter do not differ significantly from each other among plant species, according to Tukey's test ($p<0.01$); For mycelial growth ARE concentrations: 50% (dark full colour); 75% (colourless); na- not statistical analysed; Bars on the positive side of the axis - inhibitory activity; Bars on the negative side of the axis - increased activity.

On the other hand, the number of propagules observed in some Poaceae species (*L. rigidum, B. dystachion and H. murinum*) and in Brassicaceae was low (Table 3), which could be a good indicator of soil inoculum reduction (chlamydospores and zoospores).

The low density of *P. cinnamomi* detected in the soil (20 cfu/g) cultivated with *L. rigidum* seems to be in oposition with the results obtained in the bioassays *in vitro* (increase of sporangia and zoospores), which could be attributed to the presence of bacteria. The plates inoculated with that soil were fully colonized by bacteria which could have a detrimental effect on the development of the *P. cinnamomi* colonies (this assay was repeated three times).

Our results indicate that *C. arietinum, S. cereale, L. luteus* and *L. rigidum* could increase the inoculum density. In soils already infested with *P. cinnamomi* the cultivation of these species must be avoided.

DISCUSSION

Aqueous root extracts (ARE) of some species of herbaceous plants from the Mediterranean flora showed inhibitory effect on *P. cinnamomi* growth and in the production of reproductive structures. The present study intends to simulate the root exudates activity in conditions as close as possible to those occurring in nature.

It is known that, under natural conditions, sporulation of *P. cinnamomi* (sporangia production) is stimulated by soil microorganisms such as *Pseudomonas* spp. and *Chromobacterium violaceum* Schröter (Zentmyer and Marshall, 1959; Zentmyer, 1965; Zentmyer and Chen, 1969; Ayers, 1971) enhancing the production and release of zoospores and consequently increasing the infection. For the above reason a suspension of non-sterile soil (Tuset *et al*., 2001) was used in our bioassays to measure the inhibitory activity of extracts in the *P. cinnamomi* growth and in the production of the reproductive structures. The results showed that root extracts of different plant species from different families substantially varied in their inhibitory effects.

The *P. purpurea* aqueous root extract, inhibited completely the sporangia production but increased mycelial growth and the production of chlamydospores. These results are not in accordance with Neves *et al*. (2014) observations. They registered 100% of inhibition of both sporangia and chlamydospores with the *P. purpurea* ethanolic root extract. This may be due to the different methods used to obtain the root extracts.

The activity of *L. luteus* ARE showed a slight reduction of sporangia production, but potentiate the mycelial growth, an increase on chlamydospore production and the germination of zoospores. This confirms the high number of propagules detected in the soil. These results were in accordance with Serrano *et al*. (2010, 2011) confirming the possibility of this species contribute to increase inoculum potential in the soil. *Lupinus albus* was not infected by *P. cinnamomi* but its ARE increased the number of chlamydospores produced. However, the sporangia production was 100% inhibited.

Plants of the Poaceae family has been reported as showing an allelopathic effect due to their benzoxazinone content (Schulz *et al*., 2013; Tabaglio *et al*., 2013). In our study, it was observed an increase on the *P. cinnamomi* activity potentiated by the four Poaceae ARE (*B. distachyon, H. murinum, L. rigidum* and *S. cereale*). No allelopathic effect was observed, although the plants were not infected by *P. cinnamomi*. The dry weights obtained with these plants developed in the soil infested with *P. cinnamomi* was higher than those of the control (Figure 2). These data may indicate that the plants, in the presence of the pathogen, invested more in biomass production being a possible strategy of adaptation and survival (Moreira *et al*., 1999; Moreira-Marcelino, 2001). Regarding the production of sporangia, it was with the *L. rigidum* root extract (ARE) that a larger number of these structures (>100%) were produced and, therefore, a large number of viable zoospores were obtained too. The results highlighted also the possibility of pastures mix species, such as *L. luteus, L. rigidum, H. murinum,* among others, commonly used for grazing, to increase *P. cinnamomi* inoculum potential, thus contributing to increase the oak decline disease in woodlands.

In vitro, the root exudates of *D. tenuifolia* and *R. raphanistrum* showed the greatest inhibition activity on the mycelial growth of *P. cinnamomi* at both

concentrations tested. At the highest concentration both ARE showed a high inhibition activity against *P. cinnamomi* by reducing the structures formed during its life cycle and preventing the germination of zoospores. These results are in agreement with those reported by Morales-Rodríguez *et al.* (2012) and Rios *et al.* (2016) that observed an inhibitory effect of different *Brassica* spp. on the mycelial growth of *Phytophthora nicotianae* and *P. cinnamomi*, respectively.

The inhibitory effect of *B. nigra* against *P. cinnamomi* was not so effective as that achieved by *D. tenuifolia* and *R. raphanistrum*. The *Brassica nigra* ARE produced a slight inhibition on mycelial growth (18%) and a stronger inhibition on sporangia production (86%), however they did not decrease the production of chlamydospores. On the contrary, Rios *et al.* (2016) demonstrated the biofumigation effectiveness of *B. nigra* against *P. cinnamomi*. So, it is probable that *B. nigra* presents different activity according to the method applied in allelopathic control.

Brassicaceae crops were considered, in general, non-host of *P. cinnamomi*, but Krasnow and Hausbeck (2015) reported that *B. juncea* and *B. napus* were infected by *Phytophthora capsici*. Our results showed that *S. arvensis* and *B. nigra* could be infected by the *P. cinnamomi*.

The non-susceptibility of *D. tenuifolia* to *P. cinnamomi* together with the inhibitory action of its root extract, are important features demonstrated in this study. The use of *D. tenuifolia* or other plant species to control soil borne plant pathogens will be preferred as safe alternative to chemical control.

CONCLUSIONS

From the fifteen plant species of the Mediterranean flora studied, only two Brassicaceae species (*D. tenuifolia* and *R. raphanistrum*) showed a greatest inhibitory effect against *P. cinnamomi.* These species could be introduced to enrich pastures reducing the pathogen population in the soil and preventing its spread, aiming to preserve the "Montado" ecosystem.

Four other species, namely *B. nigra, L. rigidum, L. luteus* and *S. arvensis,* were colonized by *P. cinammomi.* The presence of these host plants in the "Montado" could contribute to the build up of inoculum in the soil and must therefore be avoided.

Since in general, in Portugal, oak woodlands soils infested with *P. cinnamomi* present low pH, low mineral nutrient levels and low organic matter content (Moreira and Martins, 2005) these approaches (enriched pastures with Brassicaeae and the use of non-host plants) could be applied in an integrated disease management strategy, contributing also to improve soil quality and supressiveness.

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